Sigeru DAIGOBO*: **Observations on the gametophytes of *Botrychium multifidum* from nature****

大悟法滋*：ヤマハナワラビの配偶体

Introduction Morphological studies on the gametophytes of *Botrychium* from nature have been carried out by Jeffrey (1898), Lyon (1905), Bruchmann (1906), Campbell (1921, 1922), Nishida (1954, 1955, 1956), Bierhorst (1958) and others. For the most part these descriptions have been made from the mature gametophytes with sporophytes attached. In 1972, Whittier was successful in axenic culture of the gametophytes of *B. dissectum* forma *obliquum*, and revealed that their morphology was essentially the same as that described for *B. obliquum* and *B. dissectum* from nature (Campbell, 1921; Bierhorst, 1958). Recently, similar features were observed by Gifford and Brandon (1978) for the gametophytes of *B. multifidum* grown in axenic culture. As given in details by them, the gametophytes of *B. multifidum* have never been observed in nature.

In this paper, the author describes the morphology of *B. multifidum* gametophytes from nature with various stages from very young prothallia to older mature ones.

Materials and Methods The materials of *B. multifidum* were collected by the author at the site where adult sporophytes were growing, in Sugadaira, Nagano Prefecture, in 1976 and 1978. Young sporophytes were found on the ground covered partly by *Carex lanceolata*, *Hydrocotyle ramiflora* and *Geranium thunbergii*, under the trees of *Acer rufinerve* in a stand of artificial forest. The gametophytes accompanied with sporophytes were found in the forest soil somewhat disturbed 2-3 cm deep under laying thin layer of duff. For obtaining young gametophytes without sporophytes, the patches of soil surrounding the young sporophytes, 5 cm deep approximately, were taken to the laboratory, and sifted with mesh or washed.

---

* Institute of Biological Sciences, The University of Tsukuba, Sakura-mura, Ibaraki-ken, 300-31, Japan. 筑波大学生物科学系.

** Contributions from the Sugadaira Montane Research Center, No. 51.

The gametophytes were fixed in CRAF I solution, and then embedded in paraffin for serial sections. Staining of the sections was most satisfactory when a combination of Heidenhain's hematoxylin and fast green was used.

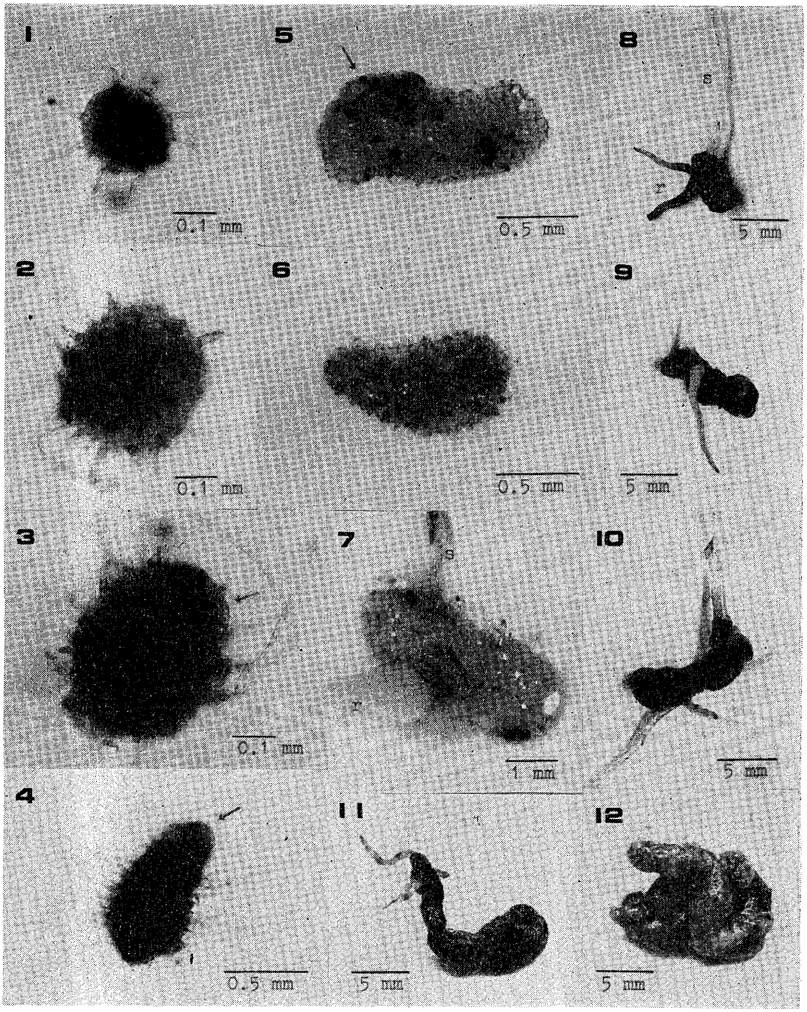
Observations About 160 gametophytes of *B. multifidum*, including 57 gametophytes with young sporophytes, have been observed in this study. They are variable in shape and size with maturation.

The youngest materials available were white and globular, measuring 0.2-0.3 mm in length (Figs. 1, 2, 24). The whole inner tissue of these prothallia was invaded with an endophytic fungus. As the gametophyte develops, it becomes ovate, 0.3-0.5 mm in length, and it can be recognized that a meristematic region is free from the fungus (Figs. 3, 23). Sometimes, an apical cell was identified from the apical meristem (Fig. 13). No sexual organs were present on these small gametophytes. These immature gametophytes provided long rhizoids arising from the surface less in number than more progressed stage.

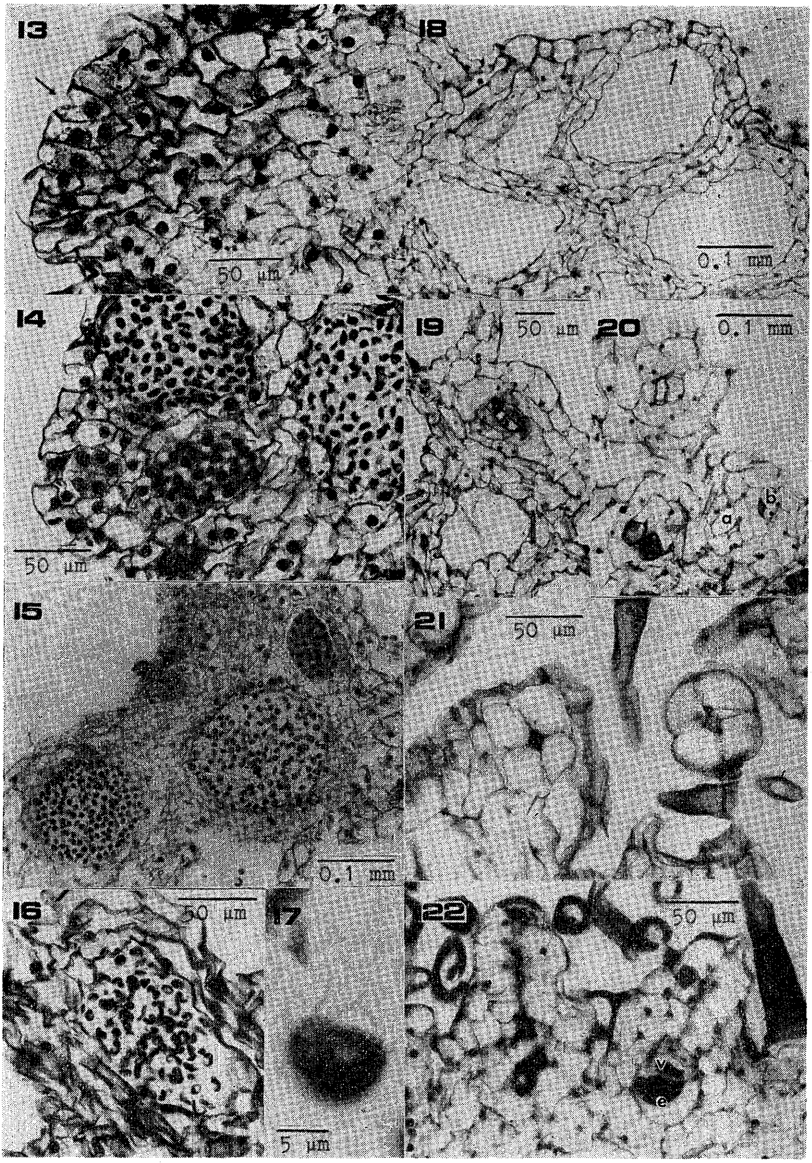
In the more advanced stage, growing up to 0.5-2 mm in length, the gametophyte becomes relatively narrower oval, and forms more or less conspicuous median ridge upon which the antheridia are borne (Figs. 4-6, 26). Such gametophytes are covered with numerous rhizoids which develop in the ventral surface. The apical meristem is often slightly inclined toward the ventral surface according to the growth of antheridial ridge (Fig. 25). The tissues of the antheridial ridge are traceable to the apical meristem. At the apical end of the ridge, new antheridia are initiated from the meristematic region.

When the gametophytes have become more than 2 mm in length, archegonia appear along the antheridial ridge of the dorsal surface (Figs. 27-30). The mature gametophytes with antheridia and archegonia are usually flat tuber in shape and their outsides are light brown in color. These are usually 3-5 mm in length and about half as wide, and covered with relatively short rhizoids. They are most commonly found in the field and most of the larger gametophytes, over 3 mm in length, bear young sporophytes (Figs. 7, 8, 32). Fig. 27 shows a median longitudinal section of the typical mature gametophyte, and Figs. 28-30 show the cross sections of the others, each position corresponds with a-c in Fig. 27.

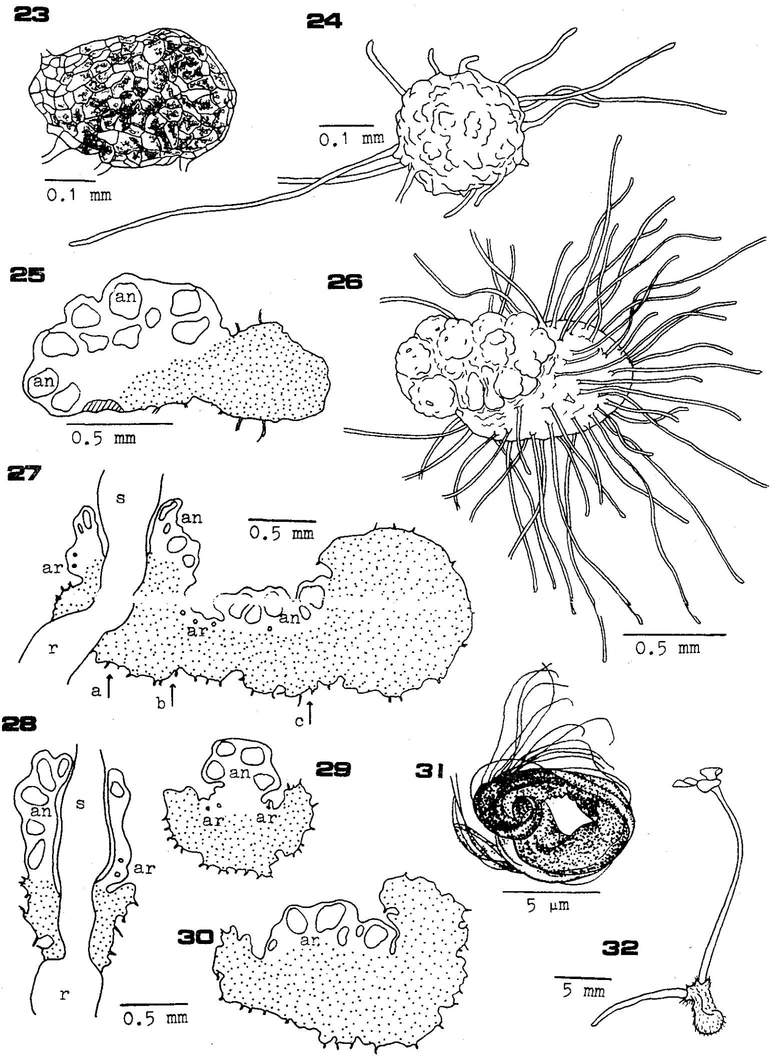
Some of the mature gametophytes are 6 to 10 mm in length, and come



Figs. 1-12. Various forms of the gametophytes of *Botrychium multifidum*. 1, 2. Very young gametophytes without sexual organs. 3. An ovate gametophyte. Arrow marks a meristematic region. 4, 5. Lateral view of young gametophytes. Arrows mark antheridial ridges. 6. Dorsal surface view of a young gametophyte. 7, 8. Mature gametophytes, showing stalk (s) and roots (r). 9-11. Large gametophytes with young sporophytes showing distorted flat tubers. 12. An abnormal gametophyte without sporophyte.



Figs. 13-22. Sections of the gametophytes of *B. multifidum*. 13. An apical meristem. Arrow marks an apical cell. 14, 15. Several stages of antheridia. 16. A mature antheridium with many spermatozoids. 17. A spermatozoid. 18. Longitudinal section of empty antheridia. Arrow marks a degenerated opercular cell. 19. Surface view of a operculum with three opercular cells. 20. Surface view of opercula, a and b showing two opercular cells of an antheridium. 21. A cross section of archegonia. 22. A longitudinal section of archegonia, showing a ventral canal cell (v) and an egg cell (e).



Figs. 23-32. Drawings of the gametophytes of *B. multifidum*. 23. Longitudinal section of a young gametophyte correspond with Fig. 3. 24. A very young gametophyte. 25. A longitudinal section of a young gametophyte, showing the antheridia (an), the extent of fungus (stippled), and the meristematic portion (shaded). 26. A young gametophyte showing the same stage of Fig. 25. 27-30. Sections of the mature gametophytes with sporophytes, showing the extent of fungus (stippled), antheridia (an), archegonia (ar), roots (r), and stalk (s). 27. A longitudinal section. 28-30. Cross sections, showing each position correspond with arrows a-c in Fig. 27. 31. A spermatozoid. 32. A mature gametophyte with young sporophyte.

up to 15-18 mm in several cases. These large gametophytes are irregular in shape, showing distorted flat tubers with bulged lower parts (Figs. 9-11). They almost have the second or third leaf and a few short rhizoids. Rarely, the large gametophyte without sporophyte has a form such a lump swirling tuberous body as indicated by Fig. 12.

The antheridia are produced usually in two or three rows on the antheridial ridge or adjacent flat areas (Figs. 26, 29, 30). Antheridia are embeded completely, or sometimes partially, in the prothallial tissue. The wall of mature antheridium consists of three-layers and rarely two layers of cells. Various stages of the development of antheridia have been observed as shown in Figs. 14, 15 and 16. However, no first series of divisions from initial cell is observed. Frequently empty antheridium with degenerated opercular cell can be found (Fig. 18). In cross section, the operculum consists of one opercular cell which is generally four-sided (Fig. 20). A second opercular cell (Fig. 20-a), or an operculum with two or three opercular cells (Fig. 19) appear in several cases.

Although special attention was paid to examine the spermatozoid in detail, only several sections showed some free spermatozoids. The spermatozoid has a somewhat coiled body with numerous cilia (Figs. 17, 31). The exact number of the cilia could not be determined.

The archegonia usually develop on the both sides of the anteridial ridge. The archegonium consists of neck cells of six to eight tiers, a neck canal cell with two nuclei, a ventral canal cell and an egg cell (Fig. 22). The neck always has four cells in cross section (Fig. 21), and protrudes straight from the surface of prothallium.

Discussion The gametophytes of *B. multifidum* are subterranian and associated with an endophytic fungus. The mature gametophyte is tuberous, somewhat flattened dorsiventrally, having a median longitudinal dorsal ridge upon which antheridia are produced. These morphological features are essentially the same as those of other *Botrychium* species so far investigated. The size of the mature gametophyte, 3-5 mm in length and about half as wide, agrees closely with that of *B. obliquum* (Campbell, 1921) or *B. dissectum* (Bierhorst, 1958).

Jeffrey (1898) reported the presence of septate rhizoids in *B. virginianum*. In *B. multifidum*, however, rhizoids are always unicellular.

According to the published data available at present, the apex of the gametophyte is composed of a small patch of meristematic cells located at or near the younger end, and usually slightly inclined towards the dorsal surface of the gametophyte. In *B. multifidum* from nature, the apex often inclined towards the ventral surface according to the growth of antheridial ridge. Such cases are also reported in *B. virginianum* and *B. dissectum* by Bierhorst (1958).

Bruchmann (1906), Bierhorst (1958) and others have given the figures of several cases in which gametophytes branched externally. Although the older prothallia often show irregular growth, no branching was found in *B. multifidum* from nature.

The tissue of gametophyte is invaded always with an endogenous fungus in every stages. The fungus is usually confined to the older tissues of the gametophyte and neither invade the meristematic tissue, nor the immediate vicinity of the reproductive organs. The fungus is densely tangled masses of clumps within the cell in structure as has been noted in other *Botrychium* species in literatures.

The gametophyte is monoecious and protandrous. The antheridia are usually produced on the dorsal median ridge, arising in acropetal succession for a long period. Although the first divisions have not been seen in the present material, the developmental pattern of antheridium seems to be similar to those reported in other species (Campbell, 1922). According to the early workers (Bruchmann, 1906; Campbell, 1921), the antheridial wall is usually two-layered and rarely three-layered, but the present author has found the latter condition to be frequent as described in *B. japonicum* by Nishida (1955).

The archegonia usually grow on the both sides of the antheridial ridge. The structure of archegonium is much like in other *Botrychium* species. Although presence of a ventral canal cell in *Botrychium* has been described as somewhat ambiguous by early workers (Campbell, 1921, 1922; Nishida, 1955), in the present study, the presence of ventral canal cell is confirmed in several archegonia.

The immature gametophytes without sexual organs from nature have been only once described in *B. obliquum* (Campbell, 1921). Whittier (1972) reported such small immature gametophytes of *B. dissectum* under some cultural conditions. The smallest grobular prothallium observed in this study is much like that of *B. obliquum* from nature, and fits the descriptions of *B. dissectum* with ovate prothallia after four months in culture except for the presence of the endophytic fungus.

Gifford and Brandon (1978) reported the morphology of gametophytes of *B. multifidum* from axenic culture. The cultivated gametophytes having a form of club-shaped with dorsal ridge are presumed to be representative of the gametophyte in nature. Actually, such form is essentially much like that of the relatively young gametophyte from nature observed in this study, but the form of the cultivated gametophyte seems to be narrower than that of wild gametophyte which swells out antheridial ridge. No archegonia have been observed in the cultivated gametophytes of *B. multifidum*, which is 2–3 mm in length, rarely 0.5 mm in length. However, the archegonia develop when the gametophytes have become more than 2 mm in length in nature.

Summary About 160 gametophytes of *B. multifidum* from nature, including various forms from very young prothallia to large mature ones, were collected and the external and internal morphology were investigated. The developmental stages of prothallia are followed by the size and shape. The wild young gametophytes have many similarities to those in cultivated *B. multifidum* described by Gifford and Brandon (1978). The anatomical features of gametophytes of *B. multifidum* from nature are essentially the same as those described for other *Botrychium* species.

Acknowledgement I would like the express my sincere thanks to Dr. Seiji Tokumasu, Sugadaira Montane Research Center, the University of Tsukuba, for his kind help in collecting the materials used in this study.

References

Bierhorst, D. W. (1958) Observations on the gametophytes of *Botrychium virginianum* and *B. dissectum*. Amer. Journ. Bot. **45**: 1-9. Bruchmann, H. (1906) Über das Prothallium und die Sporen-pflanzen von *Botrychium lunaria* Sw. Flora **96**: 203-230. Campbell, D. H. (1921) The gametophyte and embryo of *Botrychium obliquum* Mühl. Ann. Bot. **35**: 141-156. —— (1922) The gametophyte and embryo of *Botrychium simplex* Hitchcook. Ann. Bot. **36**: 441-455. Conard, H. S. (1929) Gametophyte of *Botrychium virginianum* in Iowa. Proc. Iowa Acad. Sci. **36**: 141-142. Gifford, E. M. Jr. & Brandon,

D. D. (1978) Gametophytes of *Botrychium multifidum* as grown in axenic culture. Amer. Fern J. **68**: 71-75. Jeffrey, E. C. (1898) The gametophyte of *Botrychium virginianum*. Trans. Roy. Canadian Inst. **5**: 265-294. Lyon, H. L. (1905) A new genus of Ophioglossaceae. Bot. Gaz. **40**: 455-458. Nishida, M. (1954) Gametophyte of *Botrychium japonicum* Undrew. Journ. Jap. Bot. **29**: 239-244. —— (1955) The morphology, gametophyte, young sporophyte and systematic position of *Botrychium japonicum* Und. Phytomorph. **5**: 449-456. —— (1956) The gametophyte of *Botrychium virginianum* Sw. and its endogenous fungus. Phytomorph. **6**: 67-73. Nozu, Y. (1954) The gametophyte and young sporophyte of *Botrychium japonicum* Und. Phytomorph. **4**: 430-434. Rao, L. N. (1939) A note on the gametophyte of *Botrychium virginianum* var. *lanuginosum* Sw. Curr. Sci. **3**: 119-120. Whittier, D. P. (1972) Gametophytes of *Botrychium dissectum* as grown in sterile culture. Bot. Gaz. **133**: 336-339.

* * * *

長野県菅平で採集されたヤマハナワラビの配偶体について，その外部形態と解剖学的な観察を行った。ハナワラビ属の配偶体に関するこれまでの報告は，若い胞子体をつけた成熟後の配偶体についての研究が大部分であり，若い未成熟な配偶体についての報告は数例しかない。今回採集された最小の球形の配偶体は，Whittier (1972) による *Botrychium dissectum* の培養の報告からみて，仮根形成が始ったばかりのかなり初期の配偶体と思われる。また，造卵器形成前の若い配偶体は，最近報告された Gifford and Brandon (1978) の培養によるヤマハナワラビの配偶体と比較すると，造精器が形成される背面の縦の稜の発達がいちじるしく，その生長によって分裂組織がしばしば配偶体の先端の腹面側に寄った位置に見られた。成熟した配偶体の形態は，これまで報告された数種のハナワラビ属の配偶体と基本的に同じ形態が観察されたが，造精器の壁細胞は2層でなく，西田(1955) のオオハナワラビについての報告と同じように，ほとんど3層であった。また，造卵器の腹溝細胞について，従来ハナワラビ属におけるその存在があまりはっきりとされていなかったが，今回いくつかの造卵器でその存在が観察された。

□前川文夫： **植物入門** A5版，136 pp.，さし絵白黒60，八坂書房，1979，￥1,600.

植物入門と言ってもありふれた入門書とは同一でなく，前川氏が多年に亘り蓄積したものの一部を集めたもので，興味と参考になる読物で，一つ一つが，人を引きつけずにはおかないのであるということができ，前川氏でなければ書けないと思う。八坂の他のものと共に極めて興味ある出版である。 (久内清孝)